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STRATEGIES FOR TREATING ARTERIAL RESTENOSIS USING POLYMERIC CONTROLLED RELEASE IMPLANTS

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Coronary artery obstruction is currently being treated with a number of invasive approaches involving catheter based angioplasty procedures. These have included most recently balloon angioplasty combined with expansion of obstructed coronary arteries using balloon expandable stainless steel stents. However, angioplasty itself, especially with stenting, leads to an accelerated reobstruction process, known as restenosis. Research reported in this paper has investigated an approach to preventing restenosis using controlled release drug-polymer implants for local inhibition of the pathophysiologic events of restenosis. Model therapeutic compounds were chosen including aspirin, as an antiplatelet agent, hirulog, as an antithrombin, and colchicine as an antiproliferative. Controlled release polymer matrices were successfully formulated and characterized. Retention of anticoagulant activity for the peptide, hirulog, was demonstrated *in vitro*. These polymers are suitable for investigations in periadventitial implants and animal models of restenosis. Eventually, controlled release strategies for preventing restenosis will involve integrating of ideal agents including gene therapy, with stents and related devices in order to develop a drug delivery systems approach.

INTRODUCTION

Current invasive approaches for treating coronary obstruction include balloon angioplasty, and most recently balloon angioplasty

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combined with expansion of obstructed coronary segments using balloon expendable stainless steel stents[1] to compensate for reobstruction due to elastic recoil of the arterial wall. However, the arterial stenting procedure itself induces a pronounced pathophysiologic response comparable to balloon dilatation of a diseased artery, also leading to reobstruction. This overall process has been termed restenosis. Restenosis has been the subject of a number of recent reviews.[1-3] It is known to affect up to approximately 50% of stent angioplasty procedures, within six months after stenting.[2]

The pathophysiologic mechanisms of restenosis are at present incompletely understood. Several specific events seem to predominate in the early development of the restenosis process, and these are the basis of the therapeutic strategy discussed in this paper (Table 1). Platelet thrombi form initially both along the surface of the stent itself, and the injured artery beneath it. Furthermore, without stenting, there is also subintimal exposure by balloon trauma of the vessel wall. Presumably, adherent platelets in addition to inducing subsequent fibrin thrombosis, also release important growth factors such as the "platelet derived growth factor" (PDGF), and various other active compounds such as prostaglandins, into the arterial wall, thereby influencing the subsequent proliferative response.[4] Fibrin thrombosis also contributes to acute arterial obstruction, and organization and remodeling of the fibrin thrombus can further complicate the restenosis process. A variety of growth factors and cytokines derived not only from platelets but also

Table 1. Therapeutic strategies in restenosis.

PATHOLOGIC MECHANISM	AGENT	MODE OF ACTION
Platelet Binding:		
	(a) Aspirin	(a) Prostaglandin synthesis inhibitor
	(b) Antibody to IIb/IIIa glycoprotein	(b) Blocks aggregation
	(c) Platelet derived growth factor receptor	(c) Inhibits platelet contribution to proliferation
Fibrin Clot Formation:		
	(a) Heparin*	(a) Bind to antithromb. III
	(b) Hirulog/hirudin**	(b) Binding to thrombin
Cellular Proliferation:		
	(a) Angiopeptin	(a) Inhibit smooth muscle cell proliferation
	(b) Colchicine	(b) Mitotic inhibitor
	(c) Dexamethasone	(c) Antiinflammatory steroidal effects

* Also has activity against antiplatelet Factor 4 and has antiproliferative activity.
** Inhibits thrombin binding to platelets.

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endothelial cells, smooth muscle cells, and macrophages are released at the time of vascular injury.[5] These growth factors include such well known proteins as the fibroblast growth factor, and, interleukin 1, to name a few. Proliferative events begin within the first several days after the stent-induced injury and are clearly evident experimentally within a week after stenting.

A number of strategies for ameliorating restenosis have been investigated thus far both clinically and experimentally. Clinical trials and animal protocols with various systemic drug administrations to prevent restenosis recently have been reviewed.[6] These have included investigations of aspirin (as an antiplatelet agent), heparin (an antithrombotic), colchicine (an antiproliferative), as well as a host of other agents.[6] Some of the various drugs studied experimentally thus far have included calcium channel blockers, steroids, antimetabolites such as methotrexate, and recently discovered growth antagonists such as angiopeptin.[6] In addition, coated stents have also been proposed for clinical trials.[7] The principal coating agent studied to this date has been heparin.[7] The results of all the systemic administration clinical trials, and the limited coated stent work in the clinical arena, have been thus far disappointing. No positive effects have been discovered with respect to controlling restenosis. A number of new strategies have emerged for clinical use including biodegradable stent designs, which may also include drug loadings.[8] In addition, balloon angioplasty prior to stenting with the so-called microporous or "sweating" balloon, through which agents can hopefully be seeped into the arterial wall, has also been investigated in preliminary studies clinically.[9] Spears and others have proposed combining the microporous balloon strategy with photoactivated drug use, initializing drug activity with laser energy administered through a catheter.[10]

All of the above clinical strategies have also been investigated in various animal studies, which have indicated some preliminary benefit. Most recently, studies by Edelman and his colleagues, have demonstrated that periarterial drug administration using heparin-ethylenevinyl acetate composites significantly inhibited restenosis in a rat arterial injury model.[11,12] This initial success of a controlled release drug delivery approach to restenosis has stimulated interest in the field. Controlled release drug implants have been used by our group and others to treat a variety of cardiovascular diseases, and this approach is uniquely suited for this general group of disorders.[13] Controlled release may be defined as formulations of drug polymer composites, either as monolithic matrices or reservoirs with rate limiting membrane configurations, in which drug administration can be sustained through the use of polymeric materials. Implantation of controlled release polymer systems at the site of a cardiovascular disease process offers the advantages of regional high levels of drug, with optimal drug action, as well as lowering systemic drug exposure, and thereby minimizing the possibility of side effects.

Controlled release drug administration is being used in one cardiovascular clinical application thus far, the dexamethasone releasing cardiac pacing catheter.[14] This unique application of a silicone rubber controlled release system, placed at the tip of a cardiac pacing catheter, results in inhibition of scar tissue forming near the myocardial-electrode contact site, which would otherwise raise electrical resistance, and increase pacing energy requirements. Experimental studies, by our group and others, have shown that site-specific controlled release drug implants can inhibit cardiovascular calcification and cardiac arrhythmias, delay cardiac transplant rejection, and prevent bacterial endocarditis.[15]

The goals of the present paper are as follows:

1. We will present a strategy for a mechanism-based approach to cardiovascular drug delivery for restenosis based on drugs specifically administered to selectively inhibit either the platelet component of the restenosis process, or fibrin thrombus formation, or the arterial wall proliferative response.

2. We will also describe our efforts to formulate prototypical controlled release matrices to administer regional therapy targeted at each of the above mechanisms.

3. We will present data characterizing our prototype formulations in terms of their *in vitro* bulk drug delivery and maintenance of drug activity following incorporation and release. We will also describe an animal model approach for investigating these controlled release strategies.

EXPERIMENTAL

The silicone rubber used in these studies was Dow Corning Silastic Q7-4840 (Midland, MI). The drugs used included colchicine as both a nonradioactive preparation (Sigma, St. Louis, MO), and radioactive (Tritium Labeled, New England Nuclear, Billerica, MA). Aspirin (Sigma, St. Louis, MO), hirulog as both nonradioactive and tritium labeled (Biogen, Cambridge, MA), and dexamethasone, also nonlabeled, and tritium labeled (Amersham, Arlington Heights, IL). Kits for assaying prothrombin time were obtained from Sigma, St. Louis, MO. Controlled release matrices, in general, were formulated by sieving the desired agent as a dry powder to 90 - 120 mesh particle size, and levigating it with silicone rubber prepolymer and exposing to vacuum for 30 minutes. Polymerization techniques, included casting the drug-polymer composites (20% drug, 80% polymer) into thin slabs in aluminum molds, under 20,000 psi in a Carver Press (Fisher, Chicago, IL), polymerizing at 80°C for 50 minutes. In addition, surfaces of slab matrices were sealed using the same silicone polymer in order to permit unidirectional drug release and limit swelling.

Characterization of drug release from the matrices (1X1 cm), included *in vitro* release studies in a physiologic buffer HEPES, 0.5M, pH 7.4 under perfect sink conditions for the durations required for each individual study. Retained biologic activity for the peptide anticoagulant, hirulog, was assessed using prothrombin time assays on aliquots of *in vitro* releasing buffer from the hirulog matrix studies.

RESULTS AND DISCUSSION

The agents chosen for controlled release incorporations were selected based on the mechanistic rationale described above (Table 1). Aspirin is a well known platelet antagonist, and has advantages for local release because of its acetylization of the various enzymes required for prostaglandin biosynthesis.[16] In the course of gastrointestinal administration of aspirin, hydrolysis of the molecule results in its partial deacetylization. Therefore, regional aspirin administration

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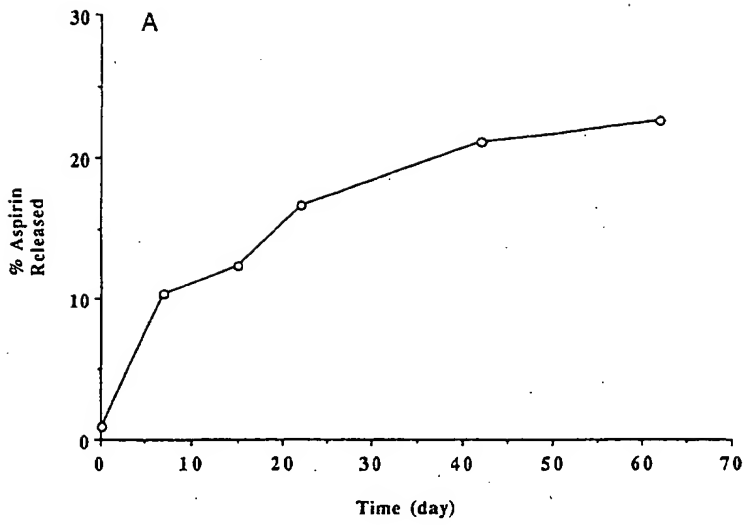
would theoretically have advantages for inhibiting prostaglandin synthesis in the arterial wall. Hirulog is a potent direct antithrombin, which binds to thrombin's substrate recognition site, and catalytic binding site. Hirulog[17] is a peptide containing the partial amino acid sequence of hirudin, the leech derived antithrombin protein. Hirulog is a potent anticoagulant, and its local administration was hypothesized to result in local anticoagulation, and thus avoid systemic anticoagulant exposure. In addition, hirulog can normally be only administered by the intravenous route, since gastrointestinal hydrolysis and absorption of the peptide would result in its deactivation. Colchicine was chosen as a mitotic inhibitor, which would hypothetically inhibit the arterial wall proliferative response.[18] Colchicine is a well known agent, which acts to inhibit cell proliferation via inhibition of microtubule formation. Dexamethasone was selected as an established antiproliferative as well as an antiinflammatory agent, with immunosuppressive properties. This agents general effects were thought to hypothetically be of benefit for many of the various components leading to the proliferative response in the arterial wall as well.

In vitro drug release results for the matrices demonstrated similar release kinetic profiles. As can be seen in Figure 1, cumulative drug release for the various compounds studied was achieved without an initial burst effect, with a nearly constant release rate, and near depletion of the matrix by 30 days of incubation.

The controlled release hirulog studies provided an opportunity to assess retention of anticoagulant activity following incorporation and release from silicone rubber polymers. A simple prothrombin time assay was used to evaluate hirulog inhibition on the prothrombin activated clot formation in this assay system. As can be seen in Table 2, throughout the duration of the *in vitro* release studies, hirulog continued to exert a significant anticoagulant activity, at activity levels comparable to standard solutions of hirulog containing the same amounts of this agent. Hirulog is also of interest, since it has antiplatelet effects. Albeit indirect, the anti-platelet effects are based on hirulog's inhibition of thrombin binding to platelets, thereby inhibiting platelet activation.[19] However, hirulog has some important limitations which are noteworthy. Hirulog is a nematode-derived peptide, and thus, sensitization with subsequent humoral immune response to this compound could potentially limit its usefulness to several weeks, and probably a one-time usage as well. In addition, controlled release investigators are just beginning to consider protein denaturation issues after incorporation, at the liquid solid interface in monolithic matrices. Hirulog, like many other peptides and proteins may be denatured due to aggregation or conformational changes or both during the time course of implantation and the progression of the drug releasing front through the monolithic matrix.

Controlled release implant strategies for preventing restenosis will have to be studied in animal models of this disorder. At present, a number of animal models are available all of which have limitations when compared to restenosis as it occurs in the human arterial wall.[6] A number of rat and rabbit models of restenosis exist, and these are based on arterial injury caused either by balloon catheter or air desiccation. The end points in these model systems are pathologic assessments after several weeks of the morphology of the arterial wall. The validity of the rat models has been questioned by many, since a number of agents which appeared to be effective in rat arterial injury models, have failed to have efficacy in larger animal models or in clinical studies.[6] Pig coronary artery stenting, followed by restenosis, is another useful model



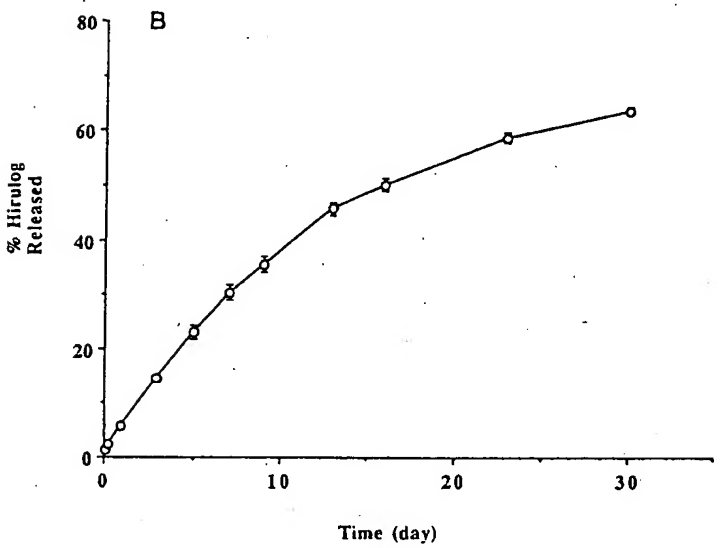
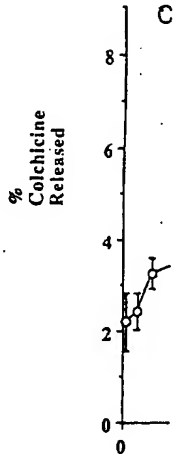


Figure 1. Cumulative release curves are shown for silicone rubber controlled release polymers for the various agents of interest. For all compounds studied, no burst phase of release was noted, and nearly constant release rates were observed for the first 20 days of drug delivery. Aspirin matrices were studied for the longest durations, and demonstrated an exponentially declining release rate with time (see A). In addition, water solubility also governed net cumulative release, as is evident for the less soluble colchicine (See 1C, opposite page, compared to A & B).



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	Prothrombin time
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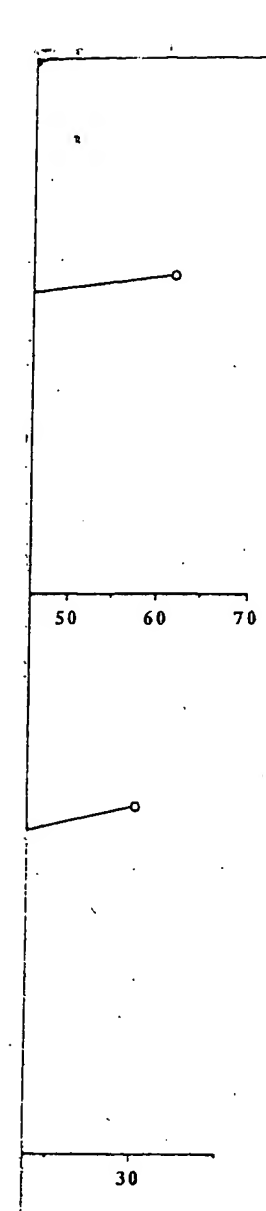

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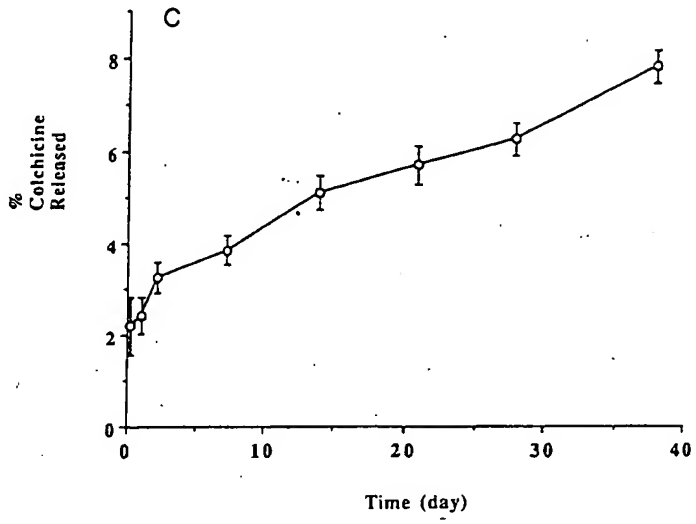

of this disorder investigated by Schwartz and others.[6,20] In these studies, pigs are subjected to coronary angioplasty, with balloon angioplasty of a coronary artery and expandable oversized stent placement. While angiography can document progression of restenosis *in vivo*, pathologic assessment of the coronary arteries is required to establish the extent of the chiefly proliferative response. In general, these studies have shown that the pig seems to have a more hyperplastic response to stenting than noted clinically. Nevertheless, the pig

Table 2. The effects of compounding and sterilization on the biological activity of Hirulog matrices

Prothrombin time test (sec); control = 8.7 ± 0.5 (mean ± SD)			
	Sterilized		Non-sterilized
	γ-irradiated*	Heat**	
Hirulog powder[a]	-	21.1 ± 2.4	21.0 ± 3.5
Hirulog[b]	12.3 ± 1.3	-	12.3 ± 1.7
Hirulog matrices released *in vitro*[c]	>45 sec (24 hr release); >115 sec (48 hr release)		
Explanted hirulog matrices after 5 days[d]	>23 (24 hr release)		

* 2 Mrads for 2 hr.
** 110°C for 30 min.
(a) 100 μg/mL.
(b) 1 cm^2; sealed, 12 hr release.
(c) unsealed.
(d) explants from pigs' advential release and released *in vitro*. $2 < n < 7$.

coronary model is probably the most comparable to the human disorder at this time. Histologically, the hyperplastic lesions produced are remarkably similar to those noted clinically following balloon angioplasty.

Another approach for studying restenosis in a larger animal model, is stent placement in the pig carotid artery.[6] This model system has been pioneered by Muller and others, and offers a number of advantages.[6] The pig carotid artery is a large and easily accessible vessel, which can be instrumented and accessed with a periadventitial controlled release matrix. Periadventitial polymer matrices are also of interest since their placement enhances intimal proliferation.[21] In addition, a number of studies have taken advantage of the fact that the contralateral carotid can be used as a control in the same animal if local strategies are to be considered.[6] Once again, the end point of this model system is typically the pathological evaluation of restenosis after a period of time following stenting, typically 30 days. However, shorter term studies can also examine the initial events in restenosis, including platelet and fibrin thrombus formation.

All of these animal model systems can be used to investigate controlled release polymer implants for restenosis. Optimal polymer configurations remain as yet to be determined. Drug delivery systems bonded to stents would be ideal, but are limited by the finite mass of the expandable stent in terms of the potential amounts of drug that could be incorporated. Periadvential drug delivery has been useful experimentally in providing adequate amounts of drug for local therapy investigations. However, periadvential controlled release would require an invasive surgical approach for clinical use, and thus alternatives to this will be needed. Polymers to be used might include biodegradable matrices, such as polylactic-polyglycolic acid, which have the advantage of disappearing without leaving a residual implant. Nondegradable matrices, such as silicone rubber or polyurethane, however have the advantage of material strength, and could in fact become part of a vascular prosthesis, if necessary, in order to maintain structural integrity in the region of the diseased blood vessel. Furthermore, nondegradable matrices could be configured as refillable drug delivery reservoirs. This design would have the advantage of replenishing drug, should long term therapy be required. The ability to use alternative agents is also a useful option in the case of reservoir implants.

Another important area of investigation for restenosis is the use of gene therapy. Pioneering work by the Nabels,[22] and by Dichek and Anderson,[23] has demonstrated effective gene transfer to the cells of the arterial wall. This has been achieved either by the use of various transection techniques to arterial wall segments, or by directly injecting genetically modified cells into an isolated arterial segment. Coating of stents and vascular grafts with genetically modified cells is also another strategy which has been successful in experimental studies. These approaches have the advantage of providing genetic coding for useful proteins, which could limit restenosis. A number of possibilities for genes encoding various proteins and peptides agents have been considered. Tissue plasminogen activator (TPA) has been successfully incorporated into endothelial cells, which have been bonded to an expandable stent and implanted into an artery in animal model studies by Anderson and Dichek.[23] TPA would presumably facilitate the clot lysis in the proximity of a stent. At this time gene therapy has a number of important limitations. Retroviral vectors for transfection of cells of the arterial wall have raised a number of concerns related to vector specificity as well as safety. Other DNA transfection techniques have focused chiefly on liposome formulations. Liposomes are limited by short

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term stability, and lack of specificity *in vivo*. In addition, all of the attempts at transfer of DNA directly to the cells of the arterial wall have been relatively inefficient, with the best results thus far achieving a transection frequency of 1% or less. Anti-sense mRNAs to proto-oncogenes have been introduced via the microporous balloon to inhibit the proliferative response in the rabbit and rat atherosclerosis models. Furthermore, gene therapy strategies can only address relatively late events in restenosis, since the time required for transcription precludes potential gene therapy of acute thrombotic events. Nevertheless, the gene therapy approach offers great promise.

Thus it can be seen that controlled release strategies for preventing restenosis will involve the integrating of ideal agents, including useful genes, with stents and related devices in order to develop a drug delivery systems approach. Ideally, if catheter based interventions are to continue to be increasingly used, drug implant interventions should be achieved by this route as well. Therefore, increasing investigative use of drug or gene loaded stents will become a priority, and their development will become an important research area.

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THE USE OF POLYICLC IN

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INTRODUCTION

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